In the Specification

Please substitute the title of the invention with the following:

~~Regulation of Systemic Immune Responses Utilizing Soluble CD40~~ Materials and Methods for Stimulating an Immune Response Using Proliferation Incompetent Cells Expressing Soluble CD40

Please amend the paragraph beginning on page 1, line 24 as follows:

Immunotherapy is a promising therapeutic approach for the treatment of cancer and is based on the premise that the failure of the immune system to reject spontaneously arising tumors is related to the failure of the immune system to appropriately respond to tumor antigens. In a functioning immune system, tumor antigens are processed and expressed on the cell surface in the context of major ~~histocompatability~~ histocompatibility complex (MHC) class I and II molecules, which are in humans also termed "human leukocyte associated" (HLA) molecules. Complexes of MHC class I and II molecules with antigenic peptides are recognized by CD8+ and CD4+ T cells, respectively. This recognition generates a set of secondary cellular signals and the paracrine release of specific cytokines or soluble so-called "biological response mediators," which mediate interactions between cells and stimulate host defenses to fight off disease. The release of cytokines then results in the proliferation of antigen-specific T cells.

Please amend the paragraph beginning on page 5, line 4 as follows:

In ~~a~~ particular embodiments of the invention, co-administration of an antigen is not required. For example, a soluble form of CD40 can be administered to an individual alone or in combination with one or more cytokines, in order to induce a systemic immune response to, for example, a pre-existent antigen, such as a poorly immunogenic tumor.

Please amend the following paragraph beginning on page 6, line 15 as follows:

Figure 10 ~~illustrate~~ illustrates vaccine therapy, Meth-A sarcoma tumors.

Please amend the paragraph beginning on page 8, line 23 as follows:

FIG. 3 illustrates natural killer cell stimulation. A, MPC11 myeloma tumor bearing ~~Balb/C~~ BALB/c mice are treated with three injections of 2 micrograms plasmid DNA encoding sCD40 (plus); GM-CSF (triangle); or luciferase (open circle) injected intra-muscular (bilaterally) and intra-tumorally using a cationic lipid transfection reagent. Plasmid DNA is injected days 4 and 8 post tumor challenge, NK assay is performed on day 11. NK activity is measured by chromium-51 release assay using YAC-1 cells as targets. Tumors are between 9.5 and 2.5 mm diameter at the time of NK assay. B, Nave mice are transfected via gene gun to the skin with 12 μg plasmid DNA, NK assay performed 3 days post transfection.

Please amend the paragraph beginning on page 9, line 1 as follows:

In FIG. 4 cytotoxic cell (CTL) assays are illustrated. A and B, syngenic mouse fibroblast cell lines are transfected with plasmid DNA encoding sCD40 (triangle); GM-CSF (diamond, ◊); G40(circle, ○); or Neo (square, □) and selected with G418. Stably transfected fibroblasts are then mixed with MPC11 tumor cells (1:1 ratio), irradiated, and used as a vaccine. Mice are vaccinated twice at one week intervals and CTL activity measured via chromium release assay 21 days after the first vaccine. C, MPCL11 myeloma cells are gene gun transfected with plasmid DNA encoding sCD40 (triangle); SM-CSF (diamond, ◊) or control (square, □), irradiated, and injected into mice and assayed as above. D, ~~Balb/C~~ BALB/c mice are challenged with J558 myeloma cells and treated with therapeutic fibroblast/J558 cell vaccine. Therapeutic vaccines, consisting of gene gun transfected irradiated mouse fibroblast cells expressing IL12/sCD40/GM-CSF mixed with J558 cells (1:1), are given on days 5 and 12 post tumor challenge. Day 35 post tumor challenge, surviving mice (40%) are sacrificed and CTL activity measured using chromium release assay. Control mice are naive age matched controls. E, C57/6 mice challenged with B16 melanoma tumor and treated via direct tumor injection of plasmid DNA encoding GM-CSF (diamond, ◊); G40 (circle, ○) or control (square, □) (10 micrograms) and cationic lipid transfection reagent 4 and 8 days post transfection. Mice are sacrificed at 14 days and CTL activity is measured by chromium release assay.

Please amend the paragraphs beginning on page 10, line 19 as follows:

In FIG. 8 vaccine therapy is illustrated, J558 myeloma tumors. ~~Balb/C~~BALB/c mice are challenged with 10.sup.6 J558myeloma cells injected intradermally on the abdomen. Therapeutic vaccine consisting of gene gun transfected irradiated mouse fibroblast cells mixed with J558 cells (1:1), are given on days 7 and 13 post tumor challenge. The first vaccine (day 7) is with fibroblast cells transfected with plasmid DNA expressing soluble CD40 and GM-CSF (triangle); GM-CSF alone (diamond) or control (square), the second vaccine (day 13) is with soluble CD40 and IL-12 (triangle; IL12 (diamond) or control (square). Each vaccination is intradermal on the abdomen bilaterally, with approximately $2x10^6$ cells per injection.

FIG. 9 illustrates vaccine therapy, MOPC myeloma tumors. ~~Balb/C~~BALB/c mice are shaved (abdomen) and challenged with 10^6 MOPC myeloma cells. Vaccine consists of 2 intradermal injections of irradiation tumor cells transfected via gene gun with plasmid DNA (2 micrograms). Mice are vaccinated 5 days post tumor challenge, with vaccine expressing sCD40 and GMOCSF (triangle); GM-CSF (diamond) or control (square), and 12 days post tumor challenge with vaccine expressing sCD40 and IL12 (triangle); IL12 (diamond) or control (square). Tumors are greater than 2 mm in diameter at the time of first vaccination.

Please amend the paragraph beginning on page 11, line 3 as follows:

FIG. 10 illustrates vaccine therapy, Meth-A sarcoma tumors. ~~Balb/C~~BALB/c mice are shaved (abdomen) and challenged with 10.sup.6 Meth-A sarcoma cells. Vaccine consists of 2 intradermal injections of irradiated tumor cells transfected via gene gun with plasmid DNA (2 micrograms). Mice are vaccinated 5 and 9 days post tumor challenge, with vaccine expressing sCD40 (triangle); GM-CSF (diamond); or control (square) tumors are greater than 5 mm in diameter at the time of the first vaccination.

Please amend the paragraph beginning on page 11, line 27 as follows:

In a preferred embodiment of the present invention, tumor cells expressing a soluble form of CD40, optionally in combination with certain cytokines, confer long term specific systemic immunity to individuals receiving such cells. In embodiments in which the cells are tumor cells, autologous cells are those which are derived from the subject and which have ~~majorhistocompatability~~ major histocompatibility (MHC) components such that such a tumor

cell obtained from a different subject would be quickly rejected. Alternatively, in other embodiments, sCD40 may be expressed from cells lacking MHC class I and class II epitopes, whereby such a line may be administered without rejection to different individual subjects. Such an approach has the advantage of not requiring biopsy and culture of a subject's own tumor cells by standard procedures.

Please amend the paragraph beginning on page 14, line 2 as follows:

The CDNA encoding the murine membrane-bound CD40 is obtained by reverse transcriptase-polymerase chain reaction (RT-PCR) of total RNA prepared from spleens of ~~Balb/c~~BALB/c mice. Extraction of RNA and RT-PCR is performed as described. A pair of primers is synthesized according to the published sequences and used for amplication of mCD40. The forward primer, 5'-GTC GCT AGC GGG CAG TGT GTT ACG TGC AGT (SEQ ID NO: 2), corresponds to nucleotides 69-89, published in the Journal of Immunology vol 148, 620-626(2) 1992, which corresponds to a site starting immediately after the putative signal peptide of the mature murine CD40 protein. This primer includes the addition of a 5' Nhel restriction enzyme site and a GTC sequence, the GTC allowing more efficient digestion of the Nhel site. The reverse primer, 5'-CTT GCT AGC ACA GAT GAC ATT AGT CTG ACT (SEQ ID NO: 3), corresponds to nucleotides 546-566 of the gene sequence published in the Journal of Immunology vol. 148, 620-626(2) 1992, which corresponds to a site starting immediately before the transmembrane domain of the mature murine CD40 protein. This primer includes the addition of a 5' Nhel restriction enzyme site and a CTT sequence, the CTT allowing more efficient digestion of the Nhel site. The final gene product encodes only the extracellular portion of the mature peptide, and excludes the signal peptide, transmembrane and cytoplasmic domains. (FIG. 2)

Please amend the paragraph beginning on page 15, line 8 as follows:

The effect of sCD40 on NK cytolytic activity in both tumor-bearing and tumor-free mice is determined. MPC myeloma tumor-bearing ~~Balb/C~~BALB/c mice are injected intramuscularly and intratumorally with 2 μg of plasmid DNA/injection encoding sCD40 or luciferase (control), using a cationic lipid transfection reagent, GenePortor. Plasmid DNA is injected on days 4 and 8 post tumor challenge. NK assay is performed on day 11. NK cytolytic assays are also performed

in nave ~~Balb/C~~BALB/c mice that had received skin transfection of plasmid vectors (12 μg of DNA each transfection). Skin transfection is performed via a gene gun. NK assay after gene gun transfection is performed 3 d later. Preparation of single-cell suspensions of splenocytes and cytolytic assays using ^{51}Cr-labeled YAC-1 target cells are performed. (FIG. 3)